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# Thermal Synthesis of Internucleotide Phosphodiester Linkages

Schramm and co-workers[1] have reported on the polymerization of mononucleotides in the presence of ethyl metaphosphate, a condensing agent which is prepared from phosphorus pentoxide and ethyl ether by refluxing with chloroform[3]. Recently, Kochetkov and co-workers[3] reported data on the structure of a polymer of uridine 2' (3')-phosphate prepared with ethyl metaphosphate (polyphosphoric ester, "PPE") in the manner of Schramm. Ribonuclease was shown to attack the polymer only to a very slight extent, and alkaline hydrolysis produced an estimated 14% of monomer (presumably uridine 2' (3')-phosphate). These authors[3] conclude that... "The above data offer unequivocal evidence in favor of the predominance of the non-natural type of linkages in polynucleotides obtained with PPE. Unfortunately, this simple method will not afford a substance useful as models of RNA in physical and biochemical studies." It is of interest, however, to examine the synthesis of such polymers not only from the point of view of their possible utility as models of RNA, but more basically as studies in the synthesis of 3' - 5' phosphodiester linkages. The present note introduces evidence that substantial amounts of phosphodiester linkages may be formed when cytidine 2'(3')-phosphate is condensed in the presence of free polyphosphoric acid.

A diagram summarizing the mode of preparation[4] is shown in Fig. 1. The ultraviolet absorption spectrum of the product is free of anomalies and shows the expected shift of the absorption maximum between acid and basic solution, indicating, as Michelson has pointed out[5], that cytosine amino groups are not involved in the internucleotide linkages. Upon treatment of the product with 0.1 M sodium hydroxide at 37° for 48 h, an increase of the extinction coefficient of approximately 18% is obtained (alkaline hyperchromicity). More important, however, is the fact that approximately 40% of the originial absorption in the ultraviolet is recovered chromatographically as cytidine 2' (3')-phosphate after such alkaline treatment. Under more extreme conditions (2.3% $NH_3$ solution, 115°, 12 h) at least 65% of the absorption may be recovered. The results of digestion of the product with bacterial alkaline

phosphatase indicate that as much as 40% of the total phosphorus is in the form of end group phosphates. This suggests a high degree of branching. However, when pancreatic ribonuclease is allowed to act on the product in conjunction with the alkaline phosphatase (Table I), almost 30% of the total in additional phosphate is liberated (that this liberation of phosphate corresponds to splitting of phosphodiester linkages is substantiated by chromatography on Sephadex G25). Since ribonuclease is specific for the 3' phosphodiester linkage, and since the starting material was a mixture of 2' and 3' cytidine phosphates, we conclude that 50 or 60% of the phosphate is present in the form of 2' or 3' phosphodiester linkages.

Researches in this laboratory in this area have centered on the relatively simple, free polyphosphoric acid as a condensing agent, as its facile synthesis by the heating of orthophosphoric acid is well known, and its possible role as an early energy source would not be unexpected. Indeed, Jones and Lipmann[6] have pointed out that the presence of inorganic polyphosphates in a great variety of organisms may represent an early means of energy distribution which was later abandoned. In this context polyphosphoric acid seems more plausible geologically than ethyl-esterified metaphosphate derivatives. The extent of characterization of thermal polynucleotides is at present much less than that of thermal heteropoly-$\alpha$-amino acids, which constituted an earlier demonstration that complex macromolecules such as a general type of protein could arise under simple thermal conditions with polyphosphoric acid[7]. Whatever the extent of chain-branching in such thermal polynucleotides, however (further structural studies are now in progress), the demonstration of significant synthesis of phosphodiester linkages, under conditions which could exist prebiologically, is of considerable interest.

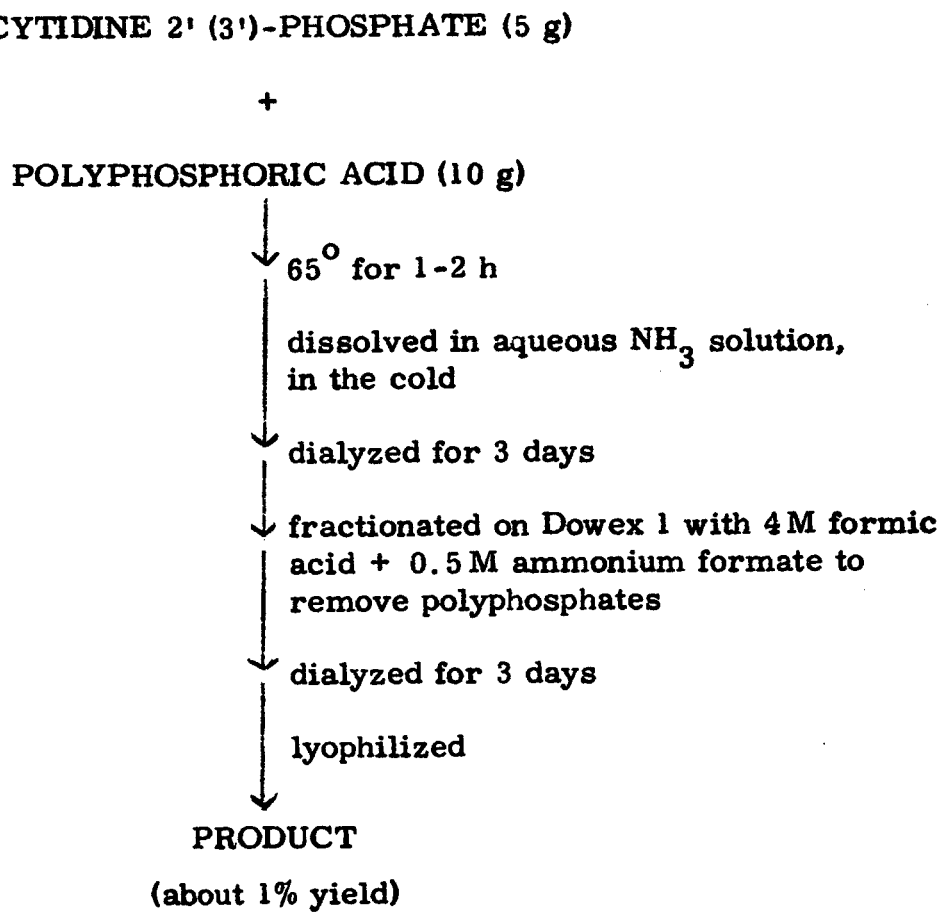


Fig. 1. Preparation and purification of a thermal product from cytidine 2' (3')-phosphate and free polyphosphoric acid.

TABLE I

Digestion of a product of cytidine 2' (3')-phosphate with alkaline phosphatase and ribonuclease

| P liberated by phosphatase (Percent of total)[1] | Additional P exposed by RNAase (Percent of total)[2] | Percent of total P accounted for |
|---|---|---|
| 36-40 | 26-29 | 88-98 |

[1]Incubation at 37° in 0.2 M sodium acetate (pH 5.3) for 4-6 h. For 5 mg of product in 10 ml of buffer, 0.5 mg of E. coli alkaline phosphatase was added.

[2]Conditions identical to 1, except that 0.5 mg of pancreatic ribonuclease was added in addition to the phosphatase.

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